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Département de médecine

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FACULTE DE MEDECINE

Professeur André CRUCHAUD

Cell-mediated lympholysis in HL-A identical unrelated individuals

THESE

présentée à la Faculté de médecine de l'Université de Genève
pour obtenir le grade de docteur en médecine

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Marc SCHAPIRA

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STEIN (Saint-Gall)

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La Faculté de médecine, sur le préavis du professeur André Cruchaud, autorise l'impression de la présente thèse, sans prétendre par là émettre d'opinion sur les propositions qui y sont énoncées.

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Cell-Mediated Lympholysis in HL-A Identical Unrelated Individuals

The mixed lymphocyte culture (MLC) reaction and the cell-mediated lympholysis (CML) represent *in vitro* models of the allograft reaction: the afferent or recognition phase is investigated by MLC and the efferent or cytotoxic phase by CML. Studies in human families (Eijsvoegel et al. 1973) have shown (1) that lymphocyte defined (LD) differences in the major histocompatibility complex (MHC) are a prerequisite for MLC activation, (2) that CML cannot occur without MLC activation, and (3) that serologically defined (SD) antigens, or antigens determined by loci very closely linked to SD loci, are the specific receptors for CML. The need for SD differences for CML was also suggested by the studies of Trinchieri et al. (1973).

Unrelated phenotypically HL-A identical, i.e. SD-identical subjects, generally stimulate in MLC (Thorsby 1973). A recent report on five SD-identical individ-

uals (Bach et al. 1973) showed that despite strong MLC activation, only one combination exhibited positive CML. We present here data on CML in five groups of HL-A-identical and MLC-positive subjects, showing a higher proportion of positive CML reactions, although the lysis is lower than between HL-A non-identical individuals.

HL-A identical individuals were selected from a panel of 800 typed healthy blood donors. At the time of the experiments, they were retyped with a battery of 180 well defined antisera directed against 27 HL-A antigens and two recently described non-HL-A lymphocyte antigens (Jeannet et al. 1973a, b). No discordant reactions were observed. Unidirectional MLC assays were done using a modification of the microtechnique described by Hartzmann et al. (1971).

In brief, the cultures contained 5×10^4 responding and 5×10^4 irradiated stimulat-

Table 1
Mixed lymphocyte culture and cell-mediated lympholysis studies in two sets of unrelated phenotypically HL-A identical subjects^a

Responder-stimulator	MLC	CML			
		⁵¹ Cr released from various target cells (cpm, mean of triplicate $\pm$ s.d.) ^c			
		Values in brackets represent percent CML $\pm$ s.d.			
		A	B	X	Y
A Br	686 $\pm$ 87	443 $\pm$ 8	520 $\pm$ 26 (6 $\pm$ 4%) [*]		
B Ar	13837 $\pm$ 4005	917 $\pm$ 55 (33 $\pm$ 5%) [*]	466 $\pm$ 35		
A Xr	49501 $\pm$ 2136	473 $\pm$ 32		922 $\pm$ 94 (40 $\pm$ 9%) ^{**}	582 $\pm$ 89 (7 $\pm$ 7%)
B Xr	69454 $\pm$ 4911		420 $\pm$ 6	1216 $\pm$ 293 (63 $\pm$ 24%) ^{**}	1158 $\pm$ 253 (47 $\pm$ 18%) ^{**}
X Yr	76713 $\pm$ 6125			456 $\pm$ 41	548 $\pm$ 53 (4 $\pm$ 5%)
Y Xr	63455 $\pm$ 3754			1391 $\pm$ 107 (77 $\pm$ 5%) ^{**}	464 $\pm$ 12
X Ar	32481 $\pm$ 6499	924 $\pm$ 67 (33 $\pm$ 5%) ^{**}	758 $\pm$ 9 (25 $\pm$ 4%) ^{**}	385 $\pm$ 19	
Y Ar	29508 $\pm$ 5232	1506 $\pm$ 105 (75 $\pm$ 9%) ^{**}	1488 $\pm$ 99 (84 $\pm$ 11%) ^{**}		509 $\pm$ 64

^a HL-A phenotype of A and B = 1, 2, 8, 17; of X and Y = 2, 3, 7, 12.

^b Control values (cpm, mean of quadruplicates $\pm$ s.e. mean): AAr = 288 $\pm$ 27; BBr = 418 $\pm$ 32; XXr = 230 $\pm$ 54; YYr = 443 $\pm$ 330.

^c ⁵¹Cr spontaneous release (SR) values (cpm, mean of 6 assays $\pm$ s.d.) and maximum release (MR) values (cpm, mean of triplicates $\pm$ s.d.): A, SR = 458 $\pm$ 27 and MR = 1852 $\pm$ 60; B, SR = 443 $\pm$ 35 and MR = 1690 $\pm$ 99; X, SR = 420 $\pm$ 48 and MR = 1677 $\pm$ 16; Y, SR = 486 $\pm$ 48 and MR = 1914 $\pm$ 85.

Significance of differences (Student's test on unpaired values) = * $P < 0.05$; ** $P < 0.001$.

ing cells (subscript "r"). The medium used was hepes-buffered Eagle's minimum essential medium, supplemented with 20 % pooled heat-inactivated AB serum and antibiotics. On the sixth day, cells were tested for incorporation of radio-labelled thymidine ($[^3\text{H}]\text{T}$) after a 16 h pulse. The CML test was performed using a modification of the method described by Lightbody et al. (1971). Aggressor cells were obtained after a 6-day co-cultivation of responding cells ($5 \times 10^5/\text{ml}$) and irradiated stimulating cells ($1.5 \times 10^5/\text{ml}$) in 20 ml medium. Target cells ($3 \times 10^5/\text{ml}$) were cultured for 6 days with phytohemagglutinin and then labelled with ^{51}Cr -chromate. CML was achieved by a 6 h incubation, at 37°C , of 1×10^6 aggressor and 2×10^4 target cells. Release of radioactivity was determined by counting the supernatant obtained after addition of phosphate-buffered saline and high speed centrifugation. Percentage of specific lysis is calculated according to the following formula:

$$\text{CML} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

Spontaneously release is determined by autolysis, i.e. the release obtained by activated aggressors confronted with autologous target cells, whereas maximum release is obtained by saponin-mediated lysis.

Results of one experiment involving two pairs of HL-A identical unrelated individuals are shown in Table 1. MLC activation, assessed by $[^3\text{H}]\text{T}$ incorporation, is high in all combinations, except in one HL-A identical pair-ABr. Otherwise there is no obvious correlation between $[^3\text{H}]\text{T}$ uptake and the number of SD antigens shared by the partners in MLC. CML is strongly positive in all combinations involving SD differences, AXr, XAr, BXr, YAr. In these cultures, the relevance of SD antigen differences for CML is confirmed by the high degree of crosslysis observed when aggressor cells are confronted with target cells sharing all SD antigens with the stimulators. In the four combinations involving HL-A identical unrelated individuals, CML is low or absent in the first

Table 2

Cell-mediated lympholysis studies in three sets of unrelated phenotypically HL-A identical subjects^a

Responding cell	Stimulating cell	Target cell	^{51}Cr release ^b (cpm, mean of triplicate $\pm$ s.d.)	CML (% $\pm$ s.d.)	P
B	A	A	520 $\pm$ 8	7 $\pm$ 2	< 0.005
A	B	B	599 $\pm$ 16	14 $\pm$ 2	< 0.001
D	C	C	263 $\pm$ 9	0 $\pm$ 1	NS
C	D	D	338 $\pm$ 6	4 $\pm$ 5	NS
F	E	E	433 $\pm$ 35	0 $\pm$ 1	NS
G	E	E	652 $\pm$ 35	9 $\pm$ 2	< 0.025
E	F	F	494 $\pm$ 46	20 $\pm$ 4	< 0.02
G	F	F	518 $\pm$ 1	22 $\pm$ 2	< 0.001
E	G	G	480 $\pm$ 28	-2 $\pm$ 2	NS
F	G	G	465 $\pm$ 35	-2 $\pm$ 2	NS

^a HL-A phenotype of A and B = 2, 9, 5, 7; of C and D = 1, 2, 5, 8; of E, F and G = 2, 3, 12, W5.

^b Control values (cpm, mean of triplicate $\pm$ s.d.); A, SR = 480 $\pm$ 7 and MR = 1290 $\pm$ 25; B, SR = 442 $\pm$ 8 and MR = 1060 $\pm$ 14; C, SR = 338 $\pm$ 6 and MR = 896 $\pm$ 25; D, SR = 263 $\pm$ 11 and MR = 1387 $\pm$ 4; E, SR = 507 $\pm$ 22 and MR = 2138 $\pm$ 43; F, SR = 256 $\pm$ 27 and MR = 2327 $\pm$ 1; G, SR = 509 $\pm$ 12 and MR = 2378 $\pm$ 67.

two (ABr tested on B target cells and XYr tested on Y) but high in the others (BAR tested on A and YXr tested on X).

Additional data, summarized in Table 2, include 10 HL-A identical combinations involving seven individuals. Out of these 10 combinations, all positive in MLC, five demonstrate a weak, but significant CML. So, out of a total of 14 combinations identical for SD but different for LD antigens, eight give positive CML, although the degree of lysis is usually less than between combinations differing for both SD and LD antigens.

If one accepts the concept that SD antigens are the specific receptors for CML (Eijssvoogel et al. 1973), two not mutually exclusive features may account for this high proportion of significant lysis: (1) the relative inaccuracy of the typing for HL-A antigens, and (2) alloimmunisation against as yet undefined SD antigens. The major sources of HL-A typing difficulties result from both the antigens and the antibodies: even well defined antigens are subject to splitting, and unsuspected heterogeneities in "monospecific" antisera have been discovered, as recently shown by population studies (Bodmer et al. 1973). Furthermore, experiments on inhibition by $F(ab')_2$ fragments of antibody-mediated complement-dependent lysis have demonstrated that antisera supposed to have the same specificity as the $F(ab')_2$ fragments gave variable results from complete to no lysis (Richiardi et al. 1973). Alloimmunisation against additional, yet undefined, SD antigens may also account for positive CML. Convincing evidence has been presented supporting the concept of a third segregant series of HL-A antigens closely associated with the FOUR series (Svejgaard et al. 1973). Furthermore, weak histocompatibility systems leading to an allograft reaction are well known in animals, and there is increasing evidence for the exis-

tence of such systems in man, as exemplified by the rejection of renal transplants or graft-versus-host reactions observed when donor and recipient are HL-A identical MLC negative siblings (Lundgren et al. 1972, Rubinstein et al. 1971). It remains to be seen whether the CML assay will be able to identify such minor histocompatibility systems as previously suggested (Wunderlich et al. 1972).

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Address:

Dr. Michel Jeannet
Unité d'Immunologie de Transplantation
Hôpital Cantonal
1211 Geneve 4
Switzerland



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